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Heat Shock Response for Ischemic Kidney Preservation and Transplantation

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The heat shock response (HSR) is a form of stress conditioning during which reversible changes in cellular metabolism are rapidly induced by brief exposure to supra-physiologic levels of heat. The nature of these adaptive adjustments has been widely investigated and has received much attention in molecular biology[1,2] and cancer research[3,4]. Recent evidence indicates that a basic form of this stress response exists at the cellular level of virtually every organism[1,5]. Although the physiological phenomenon of HSR is complex, it is well known that it can induce specific proteins, known as heat shock proteins (HSP's)[6], which are not normally synthesized. HSP's become the major proteins synthesized during the heat shock response while normal protein synthesis is suppressed. In addition, the HSR has been demonstrated to confer a transient resistance to the organism to subsequent episodes of stress[1,5].

Recently it has been reported that the HSR confers protection against cold ischemic injury and extends the cold preservation time of the rat kidney to 48 hours[7]. In this study, we have applied the concept of HSR to the preservation, and transplantation of warm ischemically injured pig kidneys. Since there is a serious shortage of cadaver kidneys available for transplantation worldwide, this number would increase if warm ischemic kidneys could be utilized. However with present methods of organ recovery and preservation, such kidneys are not likely to function after transplantation even if they were removed. We hypothesized that the application of a thermal stress to pig kidneys prior to organ procurement and preservation will enhance the organs' ability to function after warm ischemic injury.

Materials and Methods

Yorkshire pigs weighing 15 to 20 kg received halothane anesthesia. The carotid artery was cannulated for blood pressure monitoring and a Swan-Ganz catheter was placed in the iliac vein for monitoring of body core temperature. After stabilization, hemorrhagic shock was induced by the rapid withdrawal of blood from the carotid artery. After 10 minutes of hypotension (blood pressure below 50 mm Hg), the endotracheal tube was clamped and the pig was completely exsanguinated.

Key words: Heat shock response (HSR), Heat shock protein (HSP), Warm ischemic kidney, Kidney preservation, Kidney transplantation.
索引語: 熱ショック反応, 熱ショック蛋白, 温阻血腎, 腎保存, 腎移植
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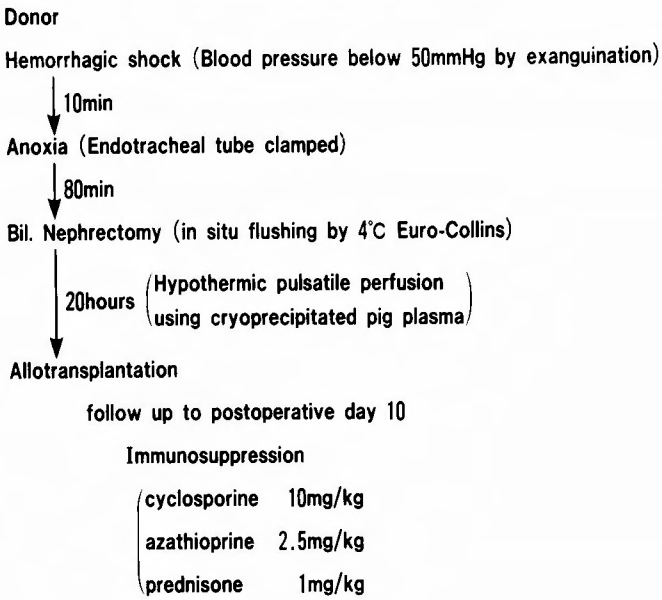
Table 1 Experimental model

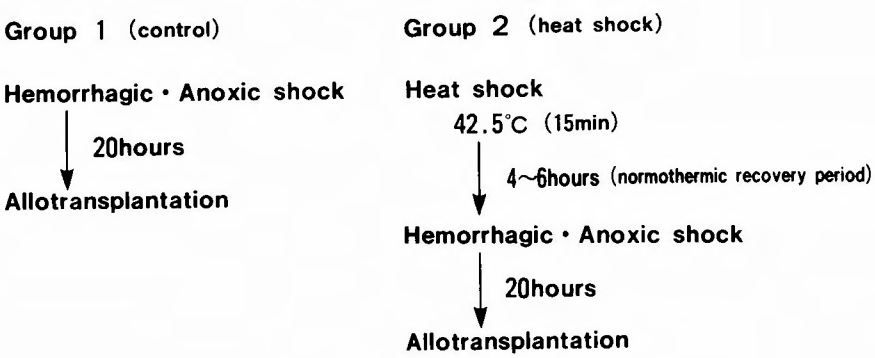


Warm ischemia was measured from the time when blood pressure fell below 50 mm Hg. Heparin (250 units/kg) and mannitol (12.5 gram) were given intravenously to all donors at the onset of hypotension. (Table 1)

Experimental groups were as follows. (Table 2) group 1 (n=8) animals received shock and warm ischemia as per the above protocol. Group 2 (n=8) animals received a operation with placement of an iliac artery to external jugular vein shunt with a heat exchanger and pump. Body temperature was risen to 42.5°C for 15 minutes using the temperature controlled heat exchanger (Fig. 1). Heating was followed by a 4–6 hour normothermic recovery period and then shock and 90 minutes of warm ischemia.

The kidneys in all groups received intra-aortic *in situ* flushing with Euro-Collins solution after the 90 minutes of warm ischemia. All kidneys were then preserved with hypothermic pulsatile perfu-

Table 2 Experimental group



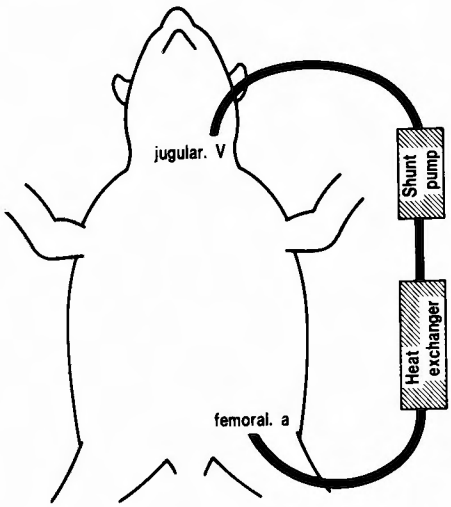


Fig. 1 Heat shock model

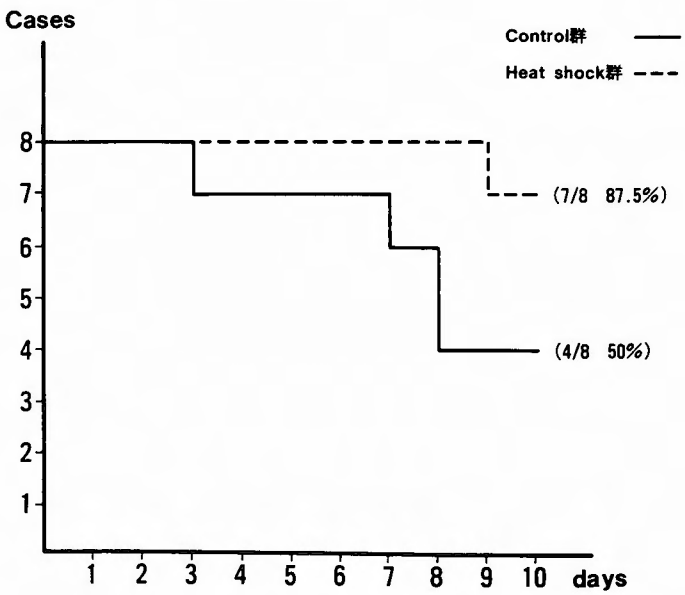


Fig. 2 Survival rate

sion using cryoprecipitated pig plasma for 20 hours followed by allotransplantation. The recipients were littermates of similar size. The transplants were placed in the right iliac fossa using end-to-end anastomosis of the renal artery to common iliac artery and end-to-side anastomosis of the renal vein to common iliac vein. The ureter was anastomosed to the bladder using a submucosal tunnel. A bilateral native nephrectomy was then performed. All pigs were given water and standard pig chow and received daily oral cyclosporine 10 mg/kg, azathioprine 2.5 mg/kg, and prednisolone 1 mg/kg beginning on the first postoperative day.

Baseline and daily postoperative serum creatinine levels were determined. Autopsies were performed on all animals at time of death or on postoperative day 10. Renal biopsy specimens were obtained from each kidney for light microscopy and were stained with hematoxylin and eosin.

Pigs with rejection and/or technical failure were excluded from the tabulation of results. A two-tailed Student's t test was used for statistical analysis.

Results

There was a higher mortality in Group 1, in which 4/8 in Group 1 died of uremia before day 10, while 7/8 in group 2 survived until sacrificed at day 10 (Fig. 2). After transplantation, kidneys in Group 1 were cyanotic and oliguric, while kidneys in Group 2 were pink and immediately diuresed. Baseline serum creatinine levels were nearly identical in both Groups, indicating similar renal function before transplantation. After transplantation, all recipients had similar creatinine elevation until postoperative day 4, when kidneys in Group 2 began to improve (Fig. 3). Mean creatinine levels on day 7: Group $1 = 11.2 \pm 4.0$ mg/dL; Group $2 = 3.7 \pm 2.7$ mg/dL. There were significant differences between Group 1 and Group 2 ($p < 0.01$) (Fig. 4). Histologic examination by light microscopy of Group 1 kidneys showed considerable cellular injury, greater than that seen in group 2 kidneys (Fig. 5).

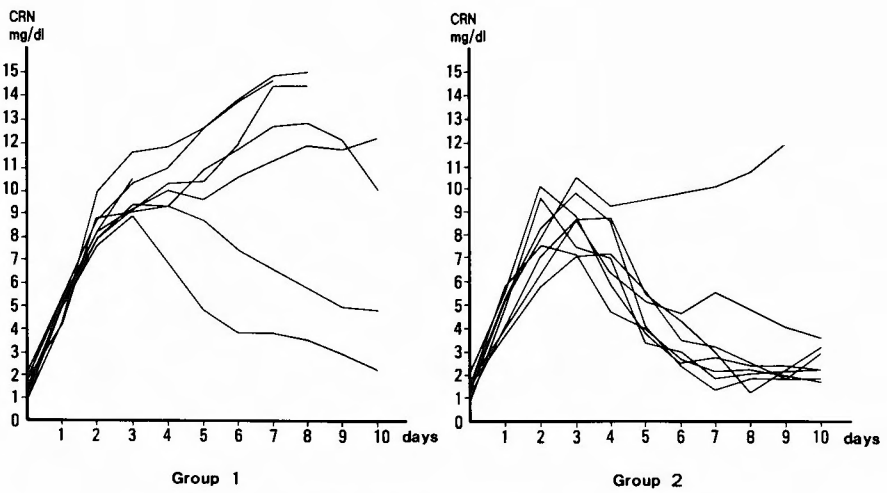


Fig. 3 Serum creatinine levels of each group

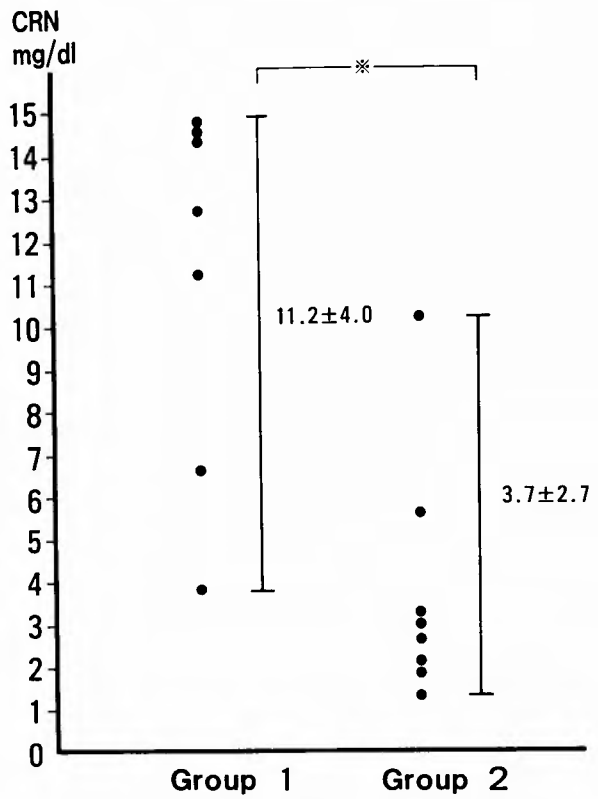


Fig. 4 Comparison of day 7 serum creatinine *$p < 0.01$

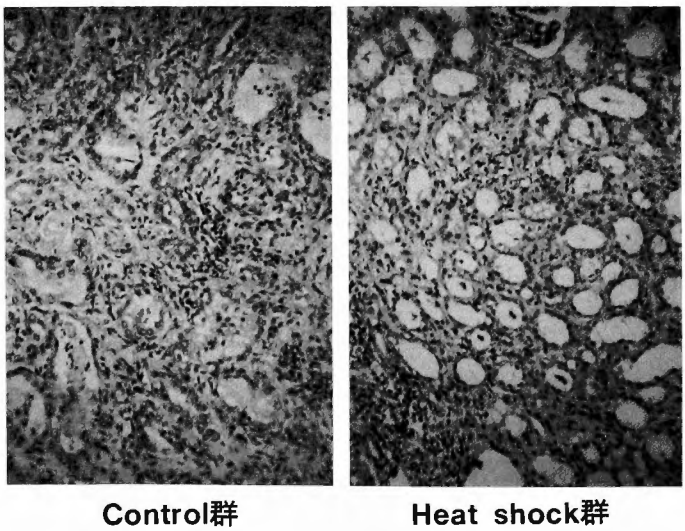


Fig. 5 Photomicrographs of sections from transplated pig kidneys previously subjected to warm ischemia and perfusion as described in text.
(Hematoxylin-eosin, original magnification 40)

Discussion

The nature of the stress response is characterized by a complex sequence of integrated events which occurs between organ systems. Recently, it has become clear that a more primitive form of stress response exists at the cellular level. The most extensively studied example of this cellular stress response is the heat shock response (HSR). The metabolic changes of the HSR are characterized by the production of a small number of highly conserved proteins termed the heat shock proteins (HSP's). Heat shock was first observed to activate specific genes in Drosophila[8], and this activation was associated with the production of a novel family of proteins[9]. Since these findings, the HSR genes have become the molecular biologist's paradigm for the study of gene activation and expression[1,10]. Features of the HSR relevant to this work are the following: 1) the HSR is universally prevalent, occurring in all species studied from bacteria to man; 2) the HSR and HSP's are highly conserved across widely disparate species, the gene and proteins associated with this phenomenon have remained remarkably similar; 3) following the induction of the HSR the cell, tissue and whole organism have now become protected against the injurious effects of the stress agent; 4) cross protection is observed, that is, the conditioning HSR can be replaced by several other forms of stress: i.e., hypoglycemia, hypoxia, and various cellular toxins[11,12,13]. These forms of stress can induce a state of thermotolerance and conversely, the HSR can induce tolerance to other forms of stress. The development of this characteristic stress response is associated with a temporary period of tolerance, during which the cell, tissue or organ is protected from an otherwise irreversible injury. This purposeful induction of cellular stress response to protect living tissues from the injurious effects of stress has recently been defined as stress conditioning[7].

Clearly, the biological responses to heat are complex and poorly understood. Nevertheless, the HSR and thermotolerance phenomena represent an inherent cellular response which is rapidly activated by stress and results in a state of temporary protection from life-threatening conditions. This translates into enhanced survival for the organism.

The ischemic damage which occurs during the agonal period of the cadaver donor has been shown to be a limiting factor for subsequent preservation and function after kidney transplantation. The injured kidneys in our experience are unlikely to function after transplantation even if they were removed with the present methods of organ recovery. The data presented shows that a conditioned whole body heating can induce substantial protection against warm ischemia. Characteristics of the stress response observed here confirm those already established by thermotolerance and the HSR phenomena. Cross protection was observed as the animals exposed to the HSR were partially protected against damage from warm ischemia. Another interesting result in our study was that a normothermic recovery time was needed for the protective benefit of HSR to occur. In basic cancer research, it has been shown that thermotolerance exists after a critical normothermic recovery time lasting anywhere between 4 and 24 hours[14]. PERDRIZET ET AL reported[7] that a 6 hour normothermic recovery period was needed to generate stress conditioning but did not observe this benefit after 0–4 hours or after 24 hours of normothermic recovery. Further studies are needed to define the optimal length of the normothermic recovery period.

The mechanism underlying the cellular stress response and resultant state of protection remains unknown. The association of stress tolerance with the appearance of the HSP's has generated much enthusiasm although an exact mechanism of action for production of these proteins remains speculative at present. Alternatively, the HSP's may represent a marker which could be easily

measured to identify "protected" or "stress conditioned" organs. Several investigators have demonstrated that the induction of thermotolerance is closely correlated with the generation of HSP's (most specifically HSP70) under different conditions[1,15].

It is well recognized that oxygen free radicals potentiate cellular injury during reperfusion after ischemia. HARVEY ET AL demonstrated[16] that warm ischemia and cold ischemia resulted in different injuries to the cell. The characteristic of warm ischemia was that continued degradation of ATP resulted in the production of xanthine which is less prevalent after cold ischemia. The reaction with xanthine oxidase results in the production of superoxide. Thus, free radicals are more likely to associate with injury when the insult is warm ischemia. Recently, it has been demonstrated that the induction of HSP could protect against reperfusion injury by a mechanism involving increased levels of catalase in the isolated rat heart model[17]. Additionally, further change exist following heat shock, including preservation of glutathione reductase level[18], alteration in membrane ATPase activity[19] and induction of superoxide dismutase activity[20]. Although the mechanism responsible for renal dysfunction due to ischemia is extremely complex, the potentially protective effect which results following recovery from heat shock might have a role in the ultimate survival of the organ.

Although clinical application of HSR is not realistic, CURRIE ET AL has demonstrated[21] that alterations in protein synthesis may occur in excised organs perfused in preparation for transplantation since those can be induced experimentally in isolated and perfused rat hearts. further study could be made utilizing stress agents other than hyperthermia which are known to be injurious to cell and tissue. Finally, the results of this study suggest that planned induction of the HSR can be used to stress condition the kidney and protect it against damage from warm ischemia. It is clear that there exists in latent form in all cells a powerful and useful protective mechanism which can be easily activated. This mechanism allows tissues in a controlled fashion, to temporarily resist subsequent injury. Through stress conditioning it is possible to provide protection in situations where stress is anticipated. The simplicity of activation of the cellular stress response would permit smooth integration into current clinical organ preservation protocols.

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和文抄録

Heat shock の阻血腎移植モデルに対する効果

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体温の上昇に伴い細胞に特有の反応がみられ，Heat shock response (HSR) と呼ばれている．このHSR が種々の侵襲に対し，一過性の生体防御機構を担っているといわれており，我々は，出血性及びAnoxic shock による温阻血腎を用いた腎移植モデルにおける HSR の効果について検討した．

雑種豚を用い，実験群を以下の2群に分けた．1群（control 群）：donor を急速脱血し血圧 50 mmHg 以下に10分間維持した後，気管内挿管チューブをクランプした anoxic shock を作製した群，2群（heat shock 群）：donor の体温を heat exchanger を用い，15分間 42.5°C に維持した後，4～6時間の常温期間をおいた後，1群同様の出血性及び Anoxic shock を作製した群とした．両群とも計90分間の温阻血後腎摘出，20時間低温持続潅流し，recipient の両側腎摘出，阻血腎を異所性に移植し，免疫抑制剤投与のもと，術後経過を行った．術後10日までの生存率は，1群 4/8 (50%)に対し，2群 7.8 (87.5%)であった．術後7日目の血清クレアチニン値は，1群 11.2±4.0 mg/*dl* に対し，2群 3.7±2.7 mg/*dl* と有意な差 ($p<0.01$) を認めた．病理学的にも，細胞障害は1群に比べ2群ではより軽度であった．

今回の実験結果では，HSR が，阻血腎に対する防御作用を認め，重度ショック豚腎の移植が可能であることが示唆された．

Heat shock により正常の蛋白の合成低下と Heat shock proteins (HSP) と呼ばれる一群の蛋白合成が誘導されることが知られている．そして，HSR の生体防御機構と HSP の合成能の消長が相関するともいわれており，今後，さらに詳しい HSR の生理学的意義とともに，HSP 自体の細胞・遺伝子レベルでの機能解析を通じて，その臨床への応用も可能なものと考えている．